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RESEARCH ARTICLE

Molecular Characterization of Bovine Rotaviruses and Coronaviruses in Diarrheic Calves in Egypt (2014-2019)

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Abstract

Bovine Rotaviruses group A (BRVA) and Bovine Coronaviruses (BCoV) are the most prevalent viral agent worldwide in diarrheic calves aged less than 6 weeks, causing economic losses due to retarded growth, increased susceptibility to other infections, treatment cost, and calf mortalities. This study aimed to detect and molecularly characterize BRVA and BCoV from diarrhetic calves. A total of 82 fecal samples were collected from calves aged less than one month from three Egyptian governorates (Alexandria, Ismailia, and Sharqia). All fecal samples were tested for BRVA and BCoV by using probe based quantitative reverse-transcription polymerase chain reaction (qRT-PCR). Consequently, positive samples contain relatively high viral genomic load were examined by RT-PCR for amplification of viral protein 7 (VP7) and viral protein 4 (VP4) genes (G- and P- typing) for BRV and full length S1 gene for BCoV. Out of 82 of tested samples, 14 (17.1%) and 22 (26.8%) were positive by qRT-PCR for BRV and BCoV, respectively. Only three and five samples had relatively high genomic load for BRV and BCoV, respectively for further testing by RT-PCR. BRV G-type was found in two samples and P-type was detected in one sample. The sequence analysis and phylogenetic tree typed these positive samples as P11 and G10. The sequences and phylogenetic analysis of BCoV positive strains (n=5) showed closely related viruses to each other and similar to previously characterized strains in Egypt since 2014. Further studies are required to antigenically characterize the circulating BRV and BCoV in Egypt.

Keywords: Rotaviruses, Coronaviruses, P typing, P11,S1 segment, Egypt.

Introduction

Acute undifferentiated calf diarrhea is a multifactorial syndrome caused by bacteria, viruses, and protozoa; these pathogens are involved in 75-95% of worldwide calf diarrheic cases. Rota and Corona viruses are the major causes of calf scour and detected in 27-36% of diseased cases[1]. There are many farm management risk factors that may exaggerate the effect of ubiquitous infectious causes of diarrhea as inadequate colostrum intake, poor calf housing, and an improper vaccination regime[2, 3].

Bovine coronavirus (BCoV) has a single stranded ribonucleic acid (ssRNA) genome that lacks proof-reading activity of the RNA-dependent RNA polymerase (RdRp), which causes low fidelity of the RNA replication machinery. This results in a high mutation rate during virus replication [4]. Additionally, coronaviruses characterized by genetic recombination [5, 6] that results in high genomic diversity[7], which consequently causes emerging of new variant that may

replace the old one and become the dominant virus [5]. BCoV cause digestive disease in young and adult cattle that result in worldwide significant economic losses in dairy and beef herds[8]. The virus is frequently involved in outbreaks of winter dysentery in adult cattle and acute undifferentiated neonatal diarrhea with varying degrees of severity in newborn calves[9-11].

Rotaviruses are non-enveloped viruses and its genome is segmented double stranded RNA, encoding structural proteins as Vp1, Vp2, Vp3, Vp4, VP6 and Vp7; and non-structural proteins as NSp1 to NSp6[12, 13]. Genetic analysis of Rotaviruses viral protein 6 (VP6) classifies the virus into 8 different groups (from A to H) [14]. Furthermore, within each group, Rotaviruses are classified into serotypes and genotypes based on antigenic and genetic variations of the VP4 and VP7. The VP7 protein is glycosylated, and its analysis classifies RVA into G groups, while VP4 is a protease sensitive polypeptide and assigns the P type groups[15]. Rotaviruses have been shown to infect wide range of young species, including infants, mammals (piglets, calves, goats, lambs, and foals) and birds [1]. Bovine rotavirus group A (BRVA) is the most prevalent viral agent worldwide in diarrheic calves aged less than 6 weeks, causing economic losses due to retarded growth, increased susceptibility to other infections, treatment costs, and calf mortalities[16].

Several studies described RVA and BCoV as major causes of acute undifferentiated calf diarrhea in Egyptian cattle and buffalo herds, causing economic losses. The BRVA was first isolated and identified in Egypt from diarrheic calves by Shalaby *et al*. [17]; furthermore, it was detected in fecal samples obtained from diarrheic calves in Ismailia, Qaluobia, and Gharbia governorates [18, 19]. Few studies described molecular characterization of BRVA and BCoV in Egypt. Merwad *et al*. [20] detected BRVA G8 type. Additionally, G6 and G10 genotypes were detected in diarrheic calves in Sharqia and Cairo governorates, respectively[21] . This study aimed to detect and molecularly characterize bovine Rota and BCoV in diarrheic calves in Egypt.

Materials and methods

Clinical samples

From September 2014 to May 2019, 12 dairy and beef farms and 13 individual cases in Ismailia, Sharqia, and Alexandria governorates complained of newborn calf diarrhea. A total of 82 diarrheic cattle and buffalo calves aged less than one month were clinically examined, and fecal samples were collected (Table 1). The samples were collected in sterilized plastic tubes and kept at -80°C till processing. Fecal samples were homogenized in equal volume of saline and 300 µL of suspension was used for RNA extraction.

Table (1): Descriptive data of the collected samples from diarrheic cattle and buffalo calves during the study

Farms	Animal species	Governorate	No. of samples	Year of collection
Farm 1	Holstein cattle	Ismailia	13	2019
Farm 2	Buffalo (Native breed)	Ismailia	6	2019
Farm 3	Buffalo (Native breed)	Ismailia	10	2019
Farm 4	Holstein cattle	Ismailia	7	2014
Farm 5	Buffalo (Native breed)	Ismailia	10	2019
Farm 6	Buffalo (Native breed)	Ismailia	5	2019
Farm 7	Buffalo (Native breed)	Ismailia	5	2019
Farm 8	Holstein cattle	Alexandria	5	2019
Farm 9	Buffalo (Native breed)	Ismailia	2	2019
Farm 10	Cattle (Native breed)	Ismailia	2	2019
Farm 11	Cattle (Native breed)	Ismailia	3	2019
Farm 12	Cattle (Native breed)	Sharqia	1	2019
Individual cases	Buffalo (Native breed)	Ismailia	6	2019
Individual cases	Buffalo (Native breed)	Ismailia	7	2019
	Total		82	

Probe based quantitative RT-PCR

Total RNA was extracted from 300 µL of fecal samples using ABT Total RNA Mini Extraction Kit (Applied biotechnology Co. Ltd., Egypt) according to manufacturer instruction. The BRV and BCV were detected by one step probe based qRT-PCR[22, 23]. Briefly, 5 µL of extracted RNA was used as template for amplification using SuperScript™ III Platinum™ One-Step qRT-PCR Kit (Invitrogen, ThermoFisher Scientific, Carlsbad, CA, USA) in Applied Biosystems™ 7500 real-time PCR system.

RT-PCR for bovine RVA typing

The positive samples by qRT-PCR with relatively high viral load (Ct value less than 20) were tested by two step hemi-nested multiplex RT-PCR systems for amplification of VP4 (P- typing) and VP7 (G typing) [24]. Briefly, 5 µl of extracted RNA was used as template for cDNA synthesis by ABT H minus cDNA synthesis kit according to manufacture instruction (Applied Biotechnology Co. Ltd, Egypt). The hemi-nested multiplex RT-PCR were performed in two rounds according to [25].

RT-PCR for amplification of S1gene of BCoV

In this study, we designed 3 overlapping primer sets to amplify the full length of S1 fragment of BCoV. Briefly, all the published genomes of BCoV in GenBank were retrieved and aligned in Genius software by Multiple Alignment using Fast Fourier Transform (MAFTT). Consequently, primers were designed from conserved regions by Integrated DNA Technologies (IDT) online software (https://eu.idtdna.com/PrimerQuest/Home /Index). The nucleotide sequences and amplicon length of all three overlapping primer sets were illustrated in (**Table 2)**. Briefly, 5 ul of extracted RNA was used as template for cDNA synthesis using ABT H-minus cDNA synthesis kit using random primers according to the manufacturers (Applied Biotechnology Co. Ltd, Egypt). Furthermore, the targeted fragments were amplified from cDNA using ABT 2X Red mix (Applied Biotechnology Co. Ltd, Egypt). Briefly, the reaction was performed in total volume of 25 uL contains 12.5 uL ABT 2X red mix, 20 picomole from each primers and 5 uL of cDNA. The cycling condition was 95°C for 3 minutes

followed by 35 cycles of 95°C for 30 seconds, 55°C for 30 seconds and 72°C for 1 minutes, then final extension at 72°C for 5 minutes. The S1 fragment of BCoV in calf guard vaccine was also amplified.

Table (2): Overlapping primer sets used for amplification of S1 subunit gene of BCoV

Primers	Sequence (5'-3')	Nucleotide position*	Sense	Amplicon size
BCoV-S1-F1	CCT GAT GTA CCY ATT TGT GTG T	23489–23510	+	986 bp
BCoV-S1-R1	ACC ATY TTG ATT GAA AGS TAG T	24453–24474	-	
BCoV-S1-F2	CAC GGT GCT YTC ACA TTA TTA	24357–24377	+	1068 bp
BCoV-S1-R2	CCT TGT AAA CAA GAR TCA ACA G	25404–25424	-	
BCoV-S1-F3	TGT TCG GGT CTT GCT ATT AAA	25324–25344	+	1115 bp
BCoV-S1-R3	TCA ACG AAA CCG ACA TCA G	26420–26438	-	

*****Oligonucleotide position is referred to the sequence of BCoV strain Mebus (GenBank accession no.: U00735.2).

DNA Sequencing and phylogenetic analysis

The PCR products were sent to Solgent Co., Ltd. (Korea) for purification and sequencing. The resulted BRV and BCoV nucleotide sequences were assembled using Geneious Software (http://www.geneious.com) and blasted to NCBI GenBank for comparison with other worldwide published sequences. The generated and blasted sequences were aligned with representative sequences from GenBank using Multiple Alignment using Fast Fourier Transform (MAFFT). The percentage of identity was calculated and phylogenetic trees were constructed using the UPGMA method [26], employing the Jukes-Cantor model. The tree topology was evaluated with 1000 bootstrap replicates.

Results

Clinical examination of diseased calves revealed loss of appetite, depression, reluctance to stand, severe watery diarrhea, dehydration and loss of body weight**.** In the first day, the feces appear watery then consequently became pasty. Its color was pale yellow to yellow green. The calf was rapidly dehydrated and recumbent. The eyes were sunken and death occur 5 days post infection.

Fecal samples testing by qRT-PCR showed that 14/82 (17.1%) and 22/82 (26.8%) samples were positive BRV and BCV, respectively.

Only three positive samples by qRT-PCR had relatively high viral load (Ct value less than 20) for amplification of BRV VP7 and VP4 genes using RT-

PCR. Only one sample was positive for VP4 (P-typing) and phylogenetic analysis typed this strain as P11 genotype (Figure 1). The nucleotide sequence was submitted to the GenBank (accession number: MN531698). For G typing, two samples were positive by RT-PCR and phylogenetic analysis typed two strains as G10 (Figure 2) (accession number: OP377078 and OP377079).

The sequence and phylogenetic analysis of VP4 showed that this positive strain is closely related to worldwide strains from Japan, China and USA with percent of identity 95.9% (Table 3) and clustered in the same branch in the phylogenetic tree (Figure 1). Furthermore,

the phylogenetic analysis of VP7 revealed that the two strains are closely related to the previously characterized strains in Egypt [21] (KX268316 and KX268317) with percentage of identity ranging from 99.1 to 99.4% (Table 4 and Figure 2).

Table (3): Identity of BRV Alex 2019 strain (P11) with other closely related strains

		1	2	3	4	5	6	7	8	9	10
ALex/2019	**1**		**94.2**	**94.2**	**95.9**	**95.9**	**95.9**	**93.6**	**95.3**	**95.3**	**93.6**
A44	**2**	**94.2**		**100**	**97.3**	**97.3**	**97.3**	**95.3**	**95.6**	**94.9**	**95.3**
Cow-tc/THA/A44/1989/G10P	**3**	**94.2**	**100**		**97.3**	**97.3**	**97.3**	**95.3**	**95.6**	**94.9**	**95.3**
B223	**4**	**95.9**	**97.3**	**97.3**		**100**	**100**	**96.9**	**96.6**	**95.9**	**95.3**
DQ-75	**5**	**95.9**	**97.3**	**97.3**	**100**		**100**	**96.9**	**96.6**	**95.9**	**95.3**
Cow-tc/USA/B223/1983	**6**	**95.9**	**97.3**	**97.3**	**100**	**100**		**96.9**	**96.6**	**95.9**	**95.3**
Yak-tc/CHN/HY-1/2018	**7**	**93.6**	**95.3**	**95.3**	**96.9**	**96.9**	**96.9**		**94.9**	**94.2**	**93.9**
Human R56/07/2007SI	**8**	**95.3**	**95.6**	**95.6**	**96.6**	**96.6**	**96.6**	**94.9**		**97.3**	**93.6**
Rabbit-/K1130027/2011	**9**	**95.3**	**94.9**	**94.9**	**95.9**	**95.9**	**95.9**	**94.2**	**97.3**		**92.9**
ARG/B2592_Co/2004	**10**	**93.6**	**95.3**	**95.3**	**95.3**	**95.3**	**95.3**	**93.9**	**93.6**	**92.9**	

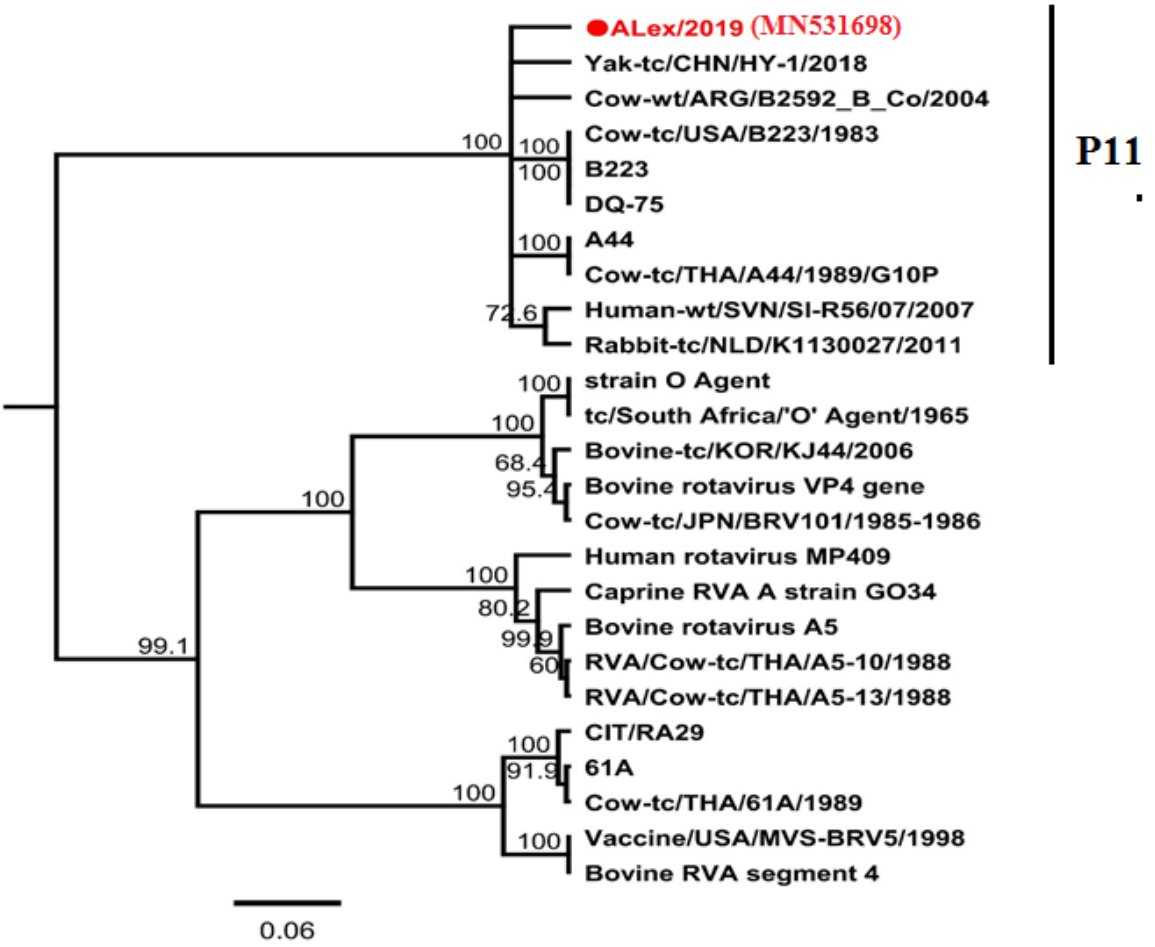

Figure (1): Phylogenetic analysis of bovine rotavirus using sequences of VP4 (P typing). The characterized strain is highlighted in red.

Table (4): Percentage of Identity of BRV Alex 2019 and Sharquia/2019 strains (G10) with other closely related strains

	Alex/2019	Sharquia/ 2019	BV-1/Sharkia/Egypt/2015 (KX268316)	BRV-2/Sharkia/Egypt/2015 (KX268317)	Human rotavirus G10P (AB714266)	VICG10.01 (GQ352366)
Alex/2019		99	99.1	99.1	94.9	94.9
Sharquia/ 2019	99		99.3	99.4	94.6	94.8
BV-1/Sharkia/Egypt/2015(KX268316)	99.1	99.3		100	94.9	95.1
BRV-2/Sharkia/Egypt/2015(KX268317)	99.1	99.4	100		94.8	95
Human rotavirus G10P(AB714266)	94.9	94.6	94.9	94.8		98.4
VICG10.01(GQ352366)	94.9	94.8	95.1	95	98.4	

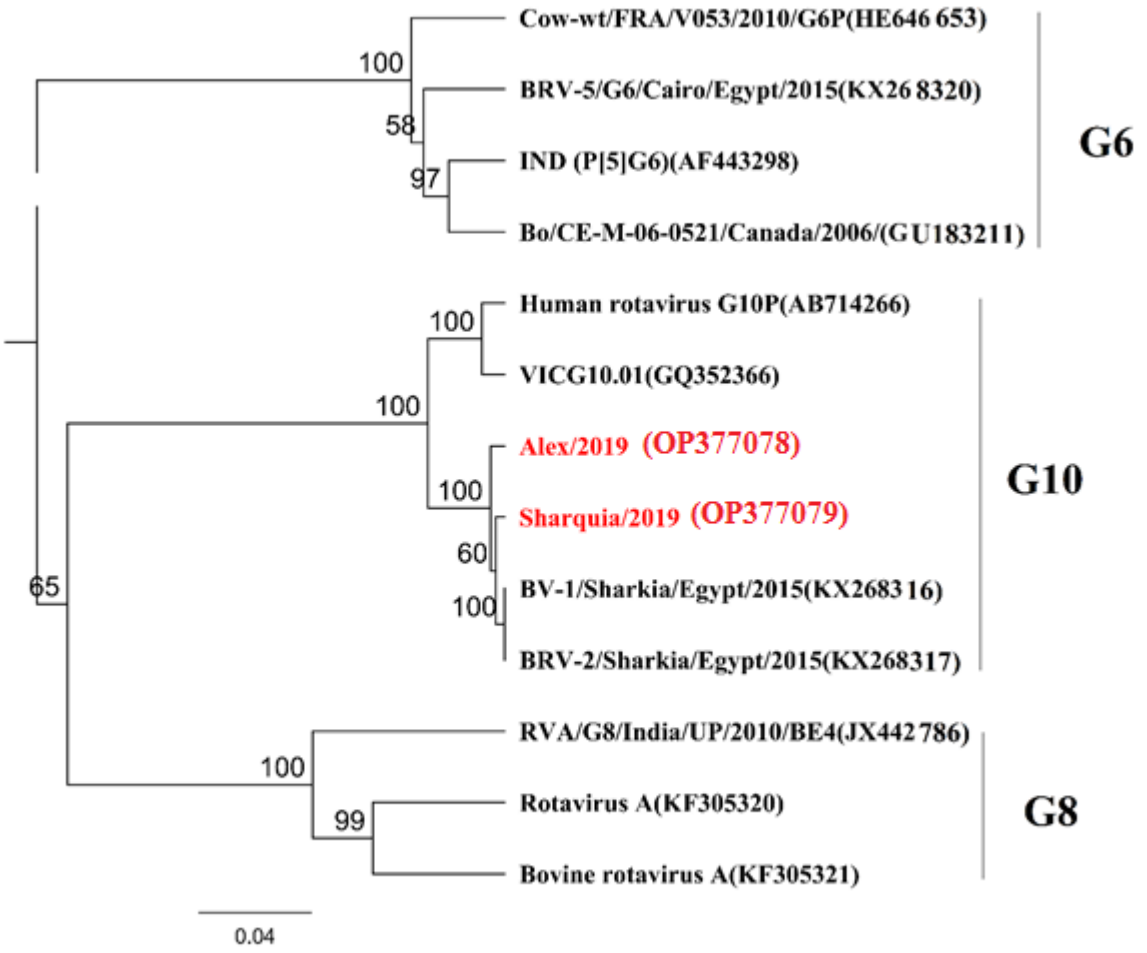

Figure (2): Phylogenetic analysis of bovine rotavirus using sequences of VP7 (G typing). The characterized strain is highlighted in red.

Five positive samples contain high viral load (Ct value less than 20) of BCoV and CALF-GUARD® vaccine by qRT-PCR were tested by RT-PCR using three overlapping primer sets for amplification of S1 segment. All tested samples showed positive reaction and give predicted amplicon size.

The sequence and phylogenetic analysis showed that the BCoV Egyptian strains 2019 were closely related to each other's and clustered in the same branch in the phylogenetic tree (Figure 3) with nucleotide identity percentages ranged (99.9 and 100%). The Egyptian 2019 strains also more similar to Egyptian strain circulated in 2014 but clustered in a separate branch with nucleotide identity percentage ranged from 98.4 to 98.5% (Table 5). The five Egyptian BCoV strains (Alex1/Egy/2019, Alex2/Egy/2019, Alex3/Egy/2019, Sharqia1/Egy/2019, and BCoV strain Ismailia/2014) and calf guard vaccine strain were submitted to GenBank with accession numbers (MN531694, MN531695, MN531696, MN531697, KM386670 and KM386671), respectively.

Table (5): Nucleotide identity and divergence of S1 gene of Egypt BCoV strains compared to prototype and vaccine strains

		1	2	3	4	5	6	7	8	9	10	11	12	13	14
BCoV strain Ismailia/ 2014	**1**		**98.5**	**98.4**	**98.4**	**98.4**	**97.4**	**97.2**	**97.5**	**98.4**	**97.5**	**97.4**	**97.4**	**97.2**	**97.4**
BCoV/ Alex1/ Egy/ 2019	**2**	**98.5**		**100**	**100**	**99.9**	**97.4**	**97.2**	**97.4**	**98.7**	**97.3**	**97.4**	**97.2**	**97.2**	**97.4**
BCoV/ Alex3/ Egy/ 2019	**3**	**98.4**	**100**		**100**	**99.9**	**97.3**	**97.1**	**97.3**	**98.7**	**97.3**	**97.4**	**97.2**	**97.1**	**97.3**
BCoV/ Alex2/ Egy/ 2019	**4**	**98.4**	**100**	**100**		**99.9**	**97.3**	**97.1**	**97.3**	**98.7**	**97.3**	**97.4**	**97.2**	**97.1**	**97.3**
BCoV/ Sharqia1/ Egy/ 2019	**5**	**98.4**	**99.9**	**99.9**	**99.9**		**97.2**	**97.1**	**97.3**	**98.6**	**97.2**	**97.3**	**97.1**	**97.1**	**97.2**
BCoV-ENT	**6**	**97.4**	**97.4**	**97.3**	**97.3**	**97.2**		**98**	**98.2**	**97.9**	**98.7**	**98.2**	**98.8**	**98**	**98.2**
Mebus	**7**	**97.2**	**97.2**	**97.1**	**97.1**	**97.1**	**98**		**99.7**	**97.6**	**97.8**	**99.6**	**98.1**	**99.7**	**99.7**
Calf guard vaccine	**8**	**97.5**	**97.4**	**97.3**	**97.3**	**97.3**	**98.2**	**99.7**		**97.9**	**98.1**	**99.9**	**98.4**	**99.7**	**99.8**
SWE_I_08-3	**9**	**98.4**	**98.7**	**98.7**	**98.7**	**98.6**	**97.9**	**97.6**	**97.9**		**97.9**	**97.8**	**97.7**	**97.6**	**97.8**
BR-UEL1	**10**	**97.5**	**97.3**	**97.3**	**97.3**	**97.2**	**98.7**	**97.8**	**98.1**	**97.9**		**98.1**	**98.6**	**97.8**	**98**
Vaccine (CoBSB)	**11**	**97.4**	**97.4**	**97.4**	**97.4**	**97.3**	**98.2**	**99.6**	**99.9**	**97.8**	**98.1**		**98.3**	**99.6**	**99.7**
KWD10	**12**	**97.4**	**97.2**	**97.2**	**97.2**	**97.1**	**98.8**	**98.1**	**98.4**	**97.7**	**98.6**	**98.3**		**98.1**	**98.3**
Quebec	**13**	**97.2**	**97.2**	**97.1**	**97.1**	**97.1**	**98**	**99.7**	**99.7**	**97.6**	**97.8**	**99.6**	**98.1**		**99.7**
L9_vaccine	**14**	**97.4**	**97.4**	**97.3**	**97.3**	**97.2**	**98.2**	**99.7**	**99.8**	**97.8**	**98**	**99.7**	**98.3**	**99.7**	

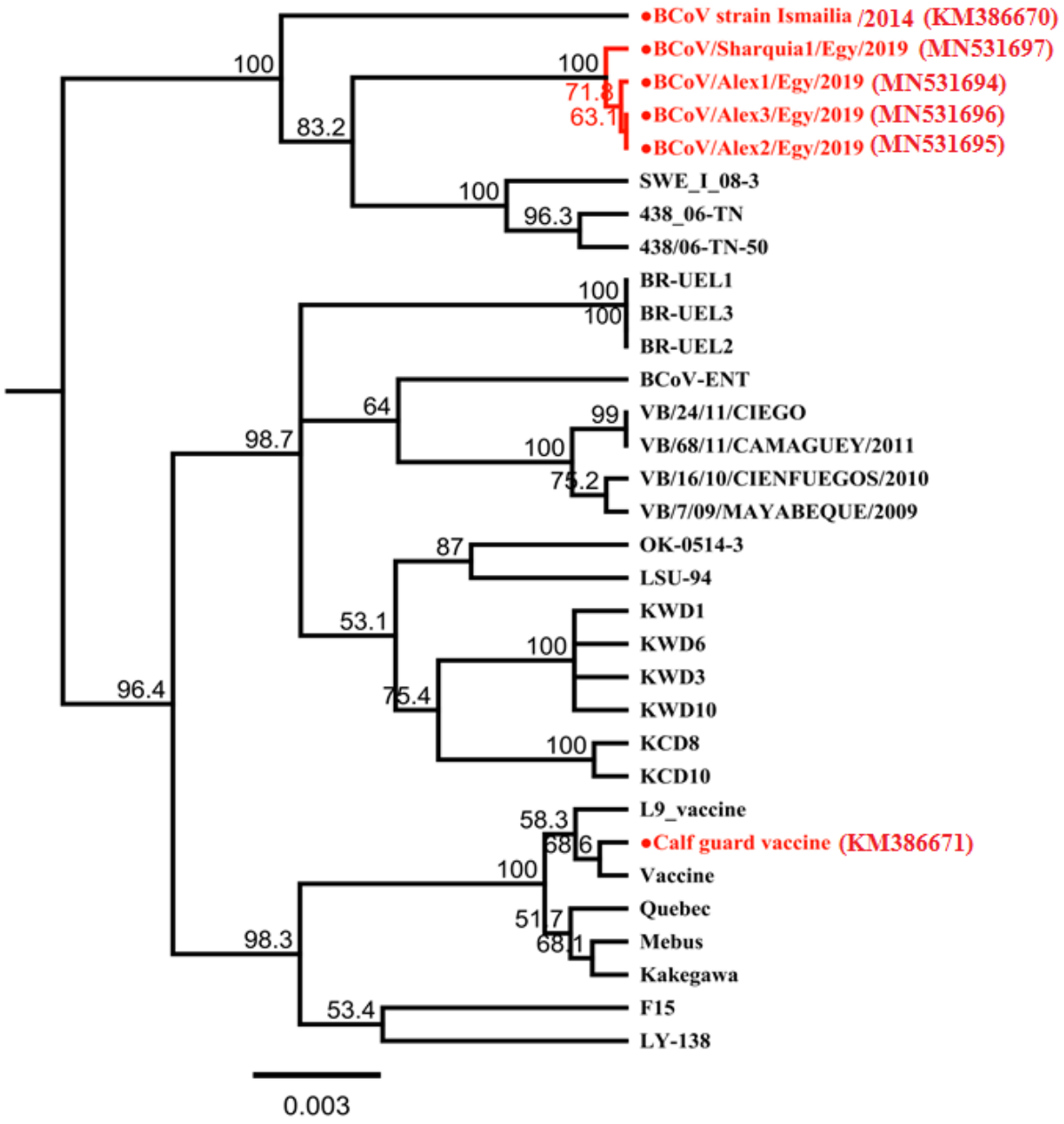

Figure (3): Phylogenetic analysis of bovine coronavirus using nucleotide sequences of S1 glycoprotein. The characterized strain is highlighted in red.

Discussion

Bovine Rota and Corona viruses are the main viral cause of neonatal diarrhea in calves; other viral causes like Bovine Viral Diarrhea virus (BVDV), Bovine Noro virus (BNoV), Bovine Astro virus (BAstV) and Bovine Toro virus (BToV) have also been implicated. Co-infection with two or more viruses and/or other bacterial, parasitic, and protozoal causes are also common and often aggravates the diarrheal symptoms[27]. It causes significant economic losses from calf mortalities, treatment costs, and reduction of weight gain in affected animals [28].

BRV infection is non-viremic and have very short incubation period (3 to 5 days) [1], while BCoV viremia has been

detected in one study[29] and the viral antigen may be detected in the small and large intestines of infected calves three weeks post infection [30].

BCoV shows a high mutation rate of about one mutation per genome per replication round[4]. In addition, a genetic recombination mechanism [5, 6], provides broad genomic diversity among CoVs [7]. Thus, over a short time period, a new variant may replace the old one and become the dominant virus [5]. Therefore, fast and accurate detection and molecular characterization of Rota and Corona viruses' infection is crucial for evaluation of control program.

Rotavirus was secreted in high viral load in fecal matter during acute infection (up to 10^{11} particle/g feces), which facilitate diagnosis by different assays as electron microscopy, antigen captured ELISA, lateral flow assays and molecular methods[25, 31-34]. The molecular methods especially qRT-PCR is characterized by higher sensitivity about two to three folds higher than other diagnostic assay [22, 35]**.**Therefore, all the collected samples in this study were tested by qRT-PCR. Fourteen (17.1%) and 22 (26.8%) samples were positive for BRV and BCoV, respectively. Our results agreed with previous study that stated higher incidence of BRV in cattle (14.50%) as compared to buffalo (8.04%) calves of 1 to 6 months of age [36]Further studies with more clinical samples may be required to figure out the role of Rota and Corona viruses in buffalo viral diarrhea.

The qRT-PCR was used for relative quantification of Rota and Corona viruses in clinical samples and selection of specimens of relatively high viral load for further testing by hemi-nested multiplex RT-PCR for molecular characterization [37]. Only three samples (Ct value less than 20) were selected for G and P- typing and only one Rotavirus strain was P typed as P11 using type specific P primers. The obtained findings agreed with other study that detected BRV P11 only in sharquia Governorate [21]. Furthermore, most worldwide studies stated that P11 is commonly detected in bovine Rota viral diarrhea[38, 39] . Two of characterized strains were typed as G and closely related to the previously characterized strains in 2011 [21].

Regarding Coronavirus typing, five positive samples (Ct value less than 20) and CALF-GUARD® vaccine by qRT-PCR were tested by hemi-nested multiplex RT-PCR for amplification of S1 segment full length. All tested samples showed positive reaction in RT-PCR and give predicted amplicons. The nucleotide sequence analysis revealed that percentage of identity between the characterized strains and vaccine isolates are 97.1 to 97.5%. The mutations in the Egyptian BCoV strain S1 subunit of S glycoprotein gene in comparison with Mebus prototype strain and Calf-guard BCoV vaccine may be attributed to the error rate of coronavirus RNA polymerase enzyme [40]. It has been reported before that S1 subunit of S glycoprotein is more sensitive to mutation [41].Our results are consistent with previous studies[42-45] that the currently circulating field strains of BCoV were genetically different in the S1 subunit of the S glycoprotein gene in comparison with the old prototype strains [43]. Also, the antigenic variability between the vaccines and currently circulating strains leads to questions about the efficacy of available vaccines. It has been previously reported that mutation in the coronavirus S glycoprotein gene results in alteration of viral antigenicity and pathogenicity [46, 47], requiring as little as a single amino acid change in the receptor binding domain in the S1 subunit of S glycoprotein to alter the virulence of the coronavirus strain [48].

Conclusion

In this study, we detected BRV and BCoV in feces of diarrhetic calves. The detected BRV were characterized as P11 and G10. Additionally, BCoV S1 segment was amplified by our developed overlapping PCR assays and characterized. Further studies with more clinical samples are required to provide more information about genetic and antigenic properties of circulating BRV and BCoV strains in Egypt.

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الملخص العربي

التوصيف الجزيئي لفيروسات الروتا البقري وفيروسات كورونا في العجول المصابة بالإسهال في مصر

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تعتبر مجموعة فيروسات الروتا من النوع A وفيروسات الكرونا من أكثر انواع الفيروسات انتشارا عالميا في العجول التي تعاني من الاسهال في اعمار تحت 6 اسابيع وتسبب خسائر اقتصادية بسبب تأخر النمو وزيادة التعرض للعدوى الأخرى وتكلفة العلاج ونفوق العجول . تهدف هذه الدراسة إلى الكشف والتوصيف الجزيئي لكل من فيروسات الروتا و الكرونا البقري من عجول الإسهال. تم تجميع 82 عينة براز من عجول تقل أعمارهم عن شهر من ثلاث محافظات مصرية (الإسكندرية ، الإسماعيلية ،و الشرقية). تم اختبار جميع عينات البراز بواسطة اختبار البلمرة المتسلسل الكمي
(qRT-PCR) لفيروسات الروتا والكرونا. وبالتالي ، تم فحص العينات الإيجابية التي تحتوي على جينوم فيروسي مرتفع نسبيًا بواسطة qRT-PCR لتضخيم جينات VP7 و VP4 (G- و typing-P) لفيروسات الروتا و RT-PCR (لتضخيم جين S1 كامل الطول) لفيروس الكرونا البقري. . كانت 14 (17.1٪) و 22 (26.8٪) عينة إيجابية من 82 عينة تم اختبارها بواسطة اختبار البلمرة المتسلسل الكمي لـفيروسات الروتا والكرونا ، على التوالي. كانت ثلاث وخمس عينات فقط تحتوي على حمل جينومي مرتفع نسبيًا لإجراء مزيد من الاختبارات بواسطة RT-PCR لفيروس الروتا والكرونا. بالنسبة للتوصيف الجيني لفيروسات الروتا ، كانت عينتان وواحدة موجبة للنوع G و P ، على التوالي. واوضح التحليل الجيني ان هذه العينات الإيجابية على أنها P11 و G10. بالنسبة للتوصيف الجيني لفيروسات الكرونا ، كانت جميع العينات الخمس المختبرة إيجابية واوضح التحليل الجيني ان الفيروسات كانت مرتبطة ارتباطًا وثيقًا ببعضها البعض وتشبه السلالة التي تم تصنيفها سابقًا في مصر عام 2014. ونوصي بالمزيد من الدراسات للتوصيف الانتجيني لفيروسات الروتا والكرونا في الابقار المنتشرة في مصر.